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Page 103, line 14, "C I 2879" should read "C I 4879."	
Page 104, line 19, "C I 4103" should read "C I 4013."	
Page 161, line 31, "A. labena n. sp." should read "Labena n. sp."	
Page 168, the cuts of figures 1 and 2 should be transposed.	
Page 229-244, throughout the paper "Pegomyia affinis Stein." should read "Pegomyia v	anduse
Mallock 1919."	
Page 248, Table III, column 2, line 11, "434" should read "334."	
Page 252, Plate 31, A, should be inverted.	
Page 260, Table I, the figures in column 2 should read, from top to bottom, "1.39, 1.94, 1.89, .24,	
.17, .19, .21, .55"; the figures in column 3 should read ".50, .10, .17, .05, .10, .11, .10, Trace, .03, .10.	.,
Page 261, line 10, "0.27" should read "0.26" and "0.17" should read "0.08."	
Page 280, paragraph 2, line 15, "41, B" should read "40, A."	
Page 285, paragraph 2, line 5, "38, D" should read "38, C."	
Page 286, paragraph 3, line 11, "tube" should read "tuber."	

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No. 1

### DETERMINATION OF ACIDITY AND TITRABLE NITRO\*GEN IN WHEAT WITH THE HYDROGEN ELECTRODE

By C. O. SWANSON, Associate Chemist, and E. L. TAGUE, Assistant Chemist, Department of Chemistry, Kansas Agricultural Experiment Station

Acidity in wheat flour is usually determined by making a water extract of the flour at a definite temperature for a definite time and titrating this extract with a standard alkali, using phenolphthalein as an indicator. Since the quantity of alkali neutralized is governed to a considerable extent by the temperature and duration of extraction, a large number of workers extract at 40° C, for two hours. This method can also be used for wheat (Triticum aesticum), the grain being first finely ground.

Eccause wheat contains the enzym phytase, as has been shown by several investigators, it is to be expected that the duration of extraction and the temperature used will influence the amount of standard alkali neutralized.

Acidity in wheat or wheat flour is not due to the presence of free acids as that term is ordinarily understood. The varying amounts of alkali neutralized in different samples are supposed to be due to the presence of phosphates in less or greater amounts. This statement is supported by the fact that the greater the acidity the greater the amount of phosphorus in the extract.<sup>2</sup>

Water extracts of wheat and wheat flour as ordinarily made are slightly turbid. This turbidity depends to some extent on the nature of the flour. Extracts from wheat and low-grade flour give clearer extracts than those from high-grade flour. This is probably due to the presence of greater amounts of electrolytes in the ground wheat and low-grade flour, as these would help to coagulate the turbid or colloidal matter.

Because of the turbidity and the colloidal nature of wheat and flour extracts, absorption plays a part in the determinations of acidity when the colorometric method is used.

 $<sup>^{-1}</sup>$  Anderson, R. J. concerning the organic phosphorus compound of wheme head and the  $\bullet$  bydrolysis of phytin. N. V. State Agr. Exp. Sta. Tech. Bul. 40, 31 p. 1915.

<sup>&</sup>lt;sup>1</sup> Swanson, C. O. Acidity in wheat flour; its relation to phosphokus and to office constituents. In Jour. Indus. and Engin. Chem., v. 4, no. 4, p. 274-278. 1912.

#### METHOD OF MAKING THE EXTRACT

A good grade of Kansas hard wheat was used for this work. The wheat was finely ground, untempered in a burr mill. Fifty gms. of this ground material were weighed into a quart Mason jar and heated to the temperature used in making the extraction. Five hundred cc. of carbon-dioxide-free water, previously heated to the temperature employed, were then added, together with 5 cc. of toulene as a preventive of bacterial action. The whole was thoroughly shaken and placed in a thermostat. The shaking was repeated every minute for the first 5 minutes, then every 15 minutes during the time of extraction. At the end of the extraction period the contents of the jar were poured into centrifuge cups and centrifuged for 5 minutes at 2,000 revolutions per minute. The supernatant liquid was then poured through a filter for the purpose of removing light floating particles. The filtrate was used for the following determinations:

- 1. The hydrogen-ion concentration or the  $P_{\rm H}$  value of the extract.
- 2. The cubic centimeters of N/2o barium hydroxid used to titrate to the absolute neutral point of  $P_{\rm H}$  7.
- The cubic centimeters of alkali used to titrate to the point of color change for phenolthalein or Pn8.3.
- The cubic centimeters of alkali used to titrate to the point of color change for thymolphthalein or Pno.3.
- The amount of alkali necessary to reneutralize after the addition of neutral formaldehyde or the Sörensen method of determining amino nitrogen.
  - 6. The total phosphorus in the extract.
- 7. The phosphorus in the extract precipitated by magnesia mixture. This may be considered phosphorus in the inorganic form. This method of determination is based on that used for the determination of inorganic phosphorus in animal tissues.

### APPARATUS USED IN DETERMINING HYDROGEN-ION CONCENTRA-

Our apparatus contains the following pieces: One Kohlrausch slide wire bridge; one type B, No. 2500 Leeds and Northrup galvanometer; one Weston millivoltmeter and multiplier; Edison storage cells; and the hydrogen and the normal calomel electrodes made according to the directions of Hildebrand. Hydrogen made by the electrolytic process was used. As a precaution against impurities, the gas was washed in a train of alkaline permanganate and pyrogallic acid.

<sup>&</sup>lt;sup>1</sup> Hildebrand, Joel H. some applications of the hydrogen electrode in analysis, researce, and teaceing. In Jour. Amen Chem. Soc., v. 35, no. 7, p. 847-871, 15 fig. 1913.

#### PERIODS OF TIME AND TEMPERATURES USED

After some preliminary work the following periods were chosen: 5, 30, and 60 minutes, 2, 4, 8, 16, and 24 hours. Extractions were made at the following temperatures: 5°, 20°, 40°, and 50° °C. Other temperatures and periods were tried in the preliminary work, but the results obtained were found of no added value.

#### METHOD OF DETERMINING THE HYDROGEN-ION CONCENTRATION

One hundred ec. of the solution prepared as above—namely, the extract of 10 gm. of ground wheat—were pipetted into the hydrogen-ion cell. This is closed with a rubber stopper through which the electrodes, as well as the tip of the burette, are inserted. In this way the carbon dioxid from the air is excluded. The hydrogen gas is bubbled through until equilibrium is reached. The reading of the voltmeter gives the figure for each lating the actual hydrogen-ion concentration of the solution. To facilitate this calculation, we have made a table giving the hydrogen-ion concentration corresponding to the volt readings from 0.281 to 1.090. It takes about an hour to obtain equilibrium, but this can be shortened by previously saturating the electrode with hydrogen.

Barium hydroxid (N/20) was then added till the solution showed a volt reading of 0.686, indicating a hydrogen-ion concentration of  $P_{\rm B}/7$ . This represents the absolute neutral point. The alkali was again added till the volt reading was 0.760, indicating a  $P_{\rm B}$  value of 8.3. This corresponds to the acidity as commonly determined by the use of phenolphthalcin as indicator. In several triads this indicator was added at this point, and gave the usual color change. The alkali was again added until the volt reading was 0.820, indicating a  $P_{\rm B}$  value of 9.3. This is the point of color change of thymolphthalcin, and this was actually determined by the use of the indicator. At this point 25 cc. of formaldehyde solution were added. This was made by mixing one part of 40 per cent formaldehyde with two parts of carbon-dioxid-free water and neutralizing to the hydrogen-ion value  $P_{\rm R}/9.3$  before using. The hydrogen gas was bubbled through until equilibrium was reached, and the  $P_{\rm B}$  value noted. Barium hydroxid (N/20) was then added until a  $P_{\rm B}$  value of 9.3 was again reached.

#### HYDROGEN-ION CONCENTRATION OF WHEAT EXTRACT

The results obtained by these methods at temperatures varying from 5° to 50° C., and for periods varying from 5 minutes to 24 hours are given in Table I.

Table I.—Hydrogen-ion concentration of wheat extracts made at different temperatures and periods, together with the quantity of N 20 barium hydroxid used in the titrating to change the hydrogen-ion concentration

			:	Quantity of N 20 barium hydroxid used to titrate to—				
Tem- pera- ture.	Time.	Volt reading.	Pa values.	o.686 or Pa 7.	o.760 or Pn 8.3.	0.820 or	adding	fernial-
°C.				Cc.	Cc.	Cc.		
5	5 minutes	0.663	6.60	0.5	I. 3	2.8	Cc.	Cc.
5	30 minutes.		6. 57	. 5	1.6	3. I	. 785	1.5
5	60 minutes	. 666	6.65	.4	1. 5	3.0	.790	1. 3
= =	24 hours	. 661	6. 57	1.5	5- 5	9.0	778	2, 2
	,	1	31	3	3.3	,,,,	.,,,	
20	5 minutes	. 662	6. 58	.8	2. I	3.3	. 763	2, 2
20	30 minutes	. 662	6. 58	1. I	3. I	4.9	. 761	2, 9
20	r hour	. 667	6. 67	1.3	4.5	7.3	. 765	3. 1
20	2 hours	. 663	6.60	1.5	5-3	8.8	. 768	3.5
20	4 hours		6. 57	2. 2	7.9	11.8	.770	3.7
20	8 hours		6. 57	3.4	9-3	13.5	. 762	4.4
20	16 hours	. 661	6. 57	3.4	14. 3	19.0	.770	4.7
20	eq hours	. 656	6. 48	4- 5	16.8	22.4	.770	4.9
	- minutos							
40	5 minutes	. 650	6. 36 6. 46	1.9	3.9	5.4	. 780	1.7
40	ı hour		6. 44	3.4	7.4	10.7	.771 .772	3-3
40	2 hours	. 643	6. 26	4.9	14. 3	10. I	762	3. 7
.10	4 hours		6.44	5.2	17.3	22.4	.772	4. 3
40	8 hours	. 653	6. 43	5.7	17. 9	23.8	.776	4. I 4. 0
40	16 hours.	. 651	6. 39	6.3	18.0	23 9	.776	4. 1
40	24 hours.		6.44	6.9	18. 2	24. I	. 780	4. 2
		İ					,	"-
50	5 minutes	. 651	6. 39	3.2	6. 5	9.0	. 762	3- 5
50	30 minutes		6.41	3-5	10.4	14.9	. 770	3.6
50	i hour	. 653	6.43	4.0	13. 7	18. 2	- 774	3. 1
50	2 hours	. 648	6. 34	5. 8	16. 3	21.4	.774	3. 2
50	4 hours	. 655	6.46	5- 3	16.4	21.5	. 780	3.0

The data in Table I show the following results:

- 1. The average hydrogen-ion concentration of the extracts obtained at 5° C. is  $P_{\rm H}$  6.60; at 20° C. it is  $P_{\rm H}$  6.59; at 40° C. it is  $P_{\rm H}$  6.43; and at 50° C. it is  $P_{\rm H}$  6.52. Thus, the higher temperature gives an extract of slightly higher hydrogen-ion concentration.
- 2. The hydrogen-ion concentration does not increase with the duration of digestion. The average volt readings at 40° C. for 5 minutes, 30 minutes, and 1 hour is 0.653, corresponding to  $P_{\rm H}$  6.43. The average volt readings for 8, 16, and 24 hours are also 0.653, corresponding to  $P_{\rm H}$  6.43. The longer or shorter period of digestion does not increase nor decrease the hydrogen-ion concentration. The slight variations obtained at 20° and 50° for the different periods are so small that they do not modify the above statement.

- 3. A volt reading of 0.760 ( $P_{\rm H}$  8.3) corresponds to the point of color change of phenolphthalein. The reading 0.820 ( $P_{\rm H}$  9.3) corresponds to the point of color change for thymolphthalein. This was determined by adding these indicators at the points mentioned.
- 4. The amount of N/20 of barium hydroxid used to titrate to  $P_{\rm H}$  8.3 is greater than the amount used to titrate to  $P_{\rm H}$  7, and the amount used to titrate to  $P_{\rm H}$  9.3 is greater than the amount used to titrate to  $P_{\rm H}$  8.3, and these differences show a progressive increase as the time of digestion is increased from 5 minutes to 24 hours.
- 5. The values obtained at 20° C, are lower than those at 40° and at 50°. At 5° they are the lowest. At this temperature very little or no hydrolysis takes place. Hydrolysis, as well as proteolysis, is most active at 10°.
- 6. The amount of N/20 barium hydroxid required to titrate to  $P_{\rm H}$  7 at  $40^{\circ}$  C. increased but little after 16 hours. In titrating to  $P_{\rm H}$  8.3 or  $P_{\rm H}$  9.3 there is very little increase after 4 hours. For example, hydrolysis is slightly slower at  $20^{\circ}$  than at  $40^{\circ}$ , and also slower at  $40^{\circ}$  than at  $50^{\circ}$ .
- 7. At the end of 4 to 8 hours at  $40^{\circ}$  C., when hydrolysis is practically complete, three times the number of cubic continueters are required to titrate to  $P_{H}$  8.3 as are necessary to titrate to  $P_{H}$  7, and four times as many are required to titrate to  $P_{H}$  9.3.
- 8. The number of cubic centimeters used to determine the titrable nitrogen after the addition of formaldehyde shows a progressive increase with the duration of digestion, but the maximum is reached in about two hours, at 40° C. At 20° the increase is much slower and continues to the end of 24 hours. At 50° the final results obtained are lower than those at either 20° or 40°, but show no increase or decrease corresponding with the time. At 5° there is very little increase from 5 minutes to 24 hours. The number of cubic centimeters required to titrate to a definite point were only about one-third as many as those required to titrate to the same point at 40°.
- 9. The most outstanding result shown in Table I is the fact that, while the hydrogen-ion concentration shows no increase with the duration of digestion, the amount of  $N/2\sigma$  barium hydroxid used to neutralize to a given hydrogen-ion concentration increases in proportion to the duration of the time of digestion. A definite limit, however, is soon reached. This limit is reached soonest at the highest temperature.
- 10. The digestion of ground wheat in water produces a substance which is not ionized; yet it will neutralize definite amounts of standard hydroxid, and these quantities correspond to a certain extent with the duration and temperature of digestion. When the standard alkali is added, it is ionized and the quantity present can be determined by the method of titration.

The results obtained with various concentrations at  $5^{\circ}$ ,  $20^{\circ}$ , and  $40^{\circ}$  C. are graphically presented in figures 1, 2, and 3.

Figure 1 shows the results of titrating to  $P_{\rm H}$  7. At 20° the increase is slow and gradual. At 40° it is very rapid up to two hours, and then it is very gradual.

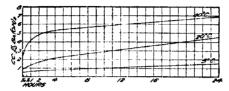


Fig. 1.—Grapus showing the hydrogen-ion concentration of wheat extract on titration to Pa 7.

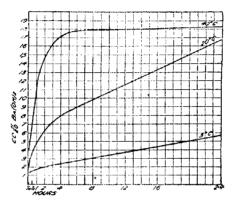


Fig. 2.—Graphs showing the hydrogen-ion concentration of wheat extract on titration to  $P_{H}$  8.3.

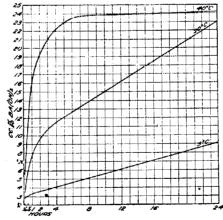


Fig. 3.—Graphs showing the hydrogen-ion concentration of wheat extract on titration to Pu 9:3.

Figure 2 shows the results of titrating to  $P_{\rm B}$  8.3. At 5° the amount of standard alkali required increases very slowly. At 20° the increase is very rapid up to four hours, followed by a gradual increase up to 24 hours. At 40° the increase is most rapid up to four hours. After that the increase is slight or practically none. The final results at 20° and 40° are not much different.

Figure 3 shows the results of titrating to P<sub>B</sub> 9.3. The general direction of the curves are similar to those in figure 2, except that the values are higher.

#### DETERMINATION OF NITROGEN IN AMINO FORM BY FORMALDEHYDE

Some investigators neutralize to litmus 1 and others to phenolphthalein 2 before adding the formaldeliyde. In Table I the results obtained by neutralizing to three points: PH 7, PH 8.3, and PH 9.3, are given. That the latter two are the points of color change for phenolphthalein and thymolphthalein, respectively, in these extracts, was determined experimentally. At PH 9.3 the formaldehyde was added and then the titration repeated until the concentration was again P<sub>B</sub> 9.3. The results of this last titration are given in the last column of Table I. The amounts given times 0.7 gives the milligrams of nitrogen in the amino form. If the formaldehyde had been added at PH 8.3 and then the titration continued to the  $P_{\rm H}$  9.3, a larger amount of N/2o barium hydroxid would have been neutralized. Still larger amounts would have been neutralized if the formaldehyde had been added at the strictly neutral point,  $P_{\rm H}$  7, and then the titration continued to the  $P_{\rm R}$  9.3 point. These amounts are given in Table II, which has been calculated from Table I.

Tribun II. "Quantity (in cubic centimeters) of N 20 barium hydroxid required had the matralization been made to the points indicated before the formaldehyde was added

Duration of extraction.	First neut	ralization t	o Pu 8.3.	First neu	tralization	to Pit 7.
	5° C.	20° C.	40° C.	5° C.	20° C.	40° C.
		-				
	Cc.	$C_{t}$ .	Cc.	Ce.	Cc.	$C_{G}$
5 ininutes	3.0	3-4	3. 2	3.8	4-7	5. 2
o minutes	3.0	4.7	6.6	4.1	6. 7	10. (
t hour	2.9	5.9	8. 3	4.0	9. 1	15.0
lours		7.0	9. I	;	10.8	18.
4 hours		7.6	9.2		13.3	21.
3 hours		8.6	9.9		15.3	22.
16 hours		9.4	10.0		• 20.3	23.
24 hours	5.7	10.5	IQ. I	9.7	22. 8	21.

<sup>&</sup>lt;sup>1</sup> ALLEN'S COMMERCIAL\*ORGANIC ANALYSIS, v. 8, p. 479. Philadelphia, 1918.

 $<sup>^{1}</sup>$  Abderhalden, Emil, ed. handbuch der biochemischen akbeitsmethoden. Bd. 3, Hällte 1, p. 228. Berlin, Wien, 1916.

The figures given under first neutralization to  $P_H$  8.3 are obtained as follows: The number of cubic centimeters of N/2o barium hydroxid obtained by titrating to  $P_H$  8.3 are subtracted from the number of cubic centimeters obtained by titrating to  $P_H$  9.3. This difference is added to the figures in the last column of Table I. The figures so obtained are assumed to be the same as if the formaldehyde had been added at the concentration  $P_H$  8.3 and then the titration resumed till the concentration  $P_H$  9.3 was obtained. The figures under first neutralization to  $P_H$  7 were calculated in the same way except that the differences between the number of cubic centimeters in the columns under  $P_H$  7 and  $P_H$  9.3 in Table I were used.

The calculations made on these assumptions show that the "formol" or titratable nitrogen obtained by first titrating to  $P_{\rm H}$  8.3 and then adding the formaldehyde is over twice that obtained by titrating first to  $P_{\rm H}$  9.3 and then adding the formaldehyde. And further, if the formaldehyde is added at  $P_{\rm H}$  7 and the titration is then continued to the concentration  $P_{\rm H}$  9.3 the amount is over four times as great.

The results obtained by this method of calculation raises the question, To what point of concentration should the solution be titrated before the formaldehyde is added? To throw light on this point an extract from wheat digested at  $40^{\circ}$  C. for four hours was used. It was prepared as the other extracts used in this investigation. This was then titrated to the points  $P_{\rm H}$  7,  $P_{\rm H}$  8.3, and  $P_{\rm H}$  9.3, first without adding any formaldehyde, and second by adding the formaldehyde before starting the titration.

Corrections were made for the differences in volume of these two. These corrections were o.r and o.2 cc. The following results were obtained (Table III).

Table III.—Quantity of N/20 sodium hydroxid required for neutralization with and without formaldehyde

Treatment ·	Quantity (in cubic centimet  N 29 sodium hydroxid u  titrate to—					
	Pn 7	Ря 8.3	Pu 9.3			
Adding the formaldehyde before starting the titration. Titrating without formaldehyde.	7. 6 4. 9	I4 5 11, 2	18. <b>0</b> 14. 6			
Increase due to formaldehyde	2. 7	3-3	3-4			

One hundred-ec. portions of extract from the wheat prepared in the same way were then used to see what differences would be obtained if the formaldehyde was added after the titration had been made to the following concentrations: P<sub>H</sub> 7, P<sub>H</sub> 8.3, and P<sub>H</sub> 9.3.

Adding Formaldehyde at Pn 7	
Total quantity (ee.) of N to sodium hydroxid to neutralize to $P_{II}(\gamma)$ . Total quantity (ee.) of N to sodium hydroxid to neutralize again to $P_{II}(\gamma)$ after adding formaldehyde.	4- 9
Adding Formaldehyde at PH 8.3	
Total quantity (cc.) of N/20 sodium hydroxid to neutralize to P <sub>H</sub>	, ,
8.3	3-3

#### Adding Formaldehyde at PH 9.3

Total quantity (cc.) of  $N/2\theta$  sodium hydroxid to neutralize to  $P_H$  14. 8 Total quantity (cc.) of  $N/2\theta$  sodium hydroxid to neutralize again to  $P_H$  9.3 after adding formaldehyde.

This shows that slightly higher results are obtained when the formaldehyde is added at the higher concentrations and the solution is titrated again to the same concentrations.

From data obtained in connection with some other work the following figures are added. The figures represent the number of cubic centimeters of N/20 alkali needed to neutralize after the addition of formaldehyde, the titrations having first been made to the concentrations shown.

Concentration.	Flour A.	Flour B.	Flour C.
The second secon			
Pn 7. Pu 8.3. Pu 9.3.	3.4	2, 5 2, 7 2, 4	3. 0 3. 1 2. 9

One question not settled by the data given in this paper is to what point should the titration be carried after the addition of the formaldehyde. This question is reserved for future work.

On the basis of the above discussion we may say that the results in Table I show the following in regard to amino nitrogen:

- 1. At 5° C, there is practically no increase in the amount of amino nitrogen as the time of digestion is increased.
- 2. At 20° C, the amount of amino nitrogen reaches the maximum shortly after 8 hours.
- 3. At 40° C, the amount of amino nitrogen reaches the maximum at 2 hours.
- 4. The calculated results in Table II show a gradual increase in the amount of amino nitrogen to the end of the 24 hours with one minor exception at 40° C. The results on amino nitrogen are graphically presented in figure 4.

#### PHOSPHORUS IN THE WHEAT EXTRACTS

As previously mentioned, determinations were made of total phosphorus and of phosphorus precipitated by magnesia mixture.

From each extraction mixture two portions of 50 cc. each, representing 5 gm. of ground wheat, were pipetted into beakers. Ten cc. of concentrated nitric acid were added and boiled until all the organic matter was destroyed, more nitric acid being added as needed. The residue was used for the determination of phosphorus in the usual way.

To two other 50-cc. portions of the extraction mixture there were added 40 cc. of magnesia mixture, and after standing for 15 minutes, 25 cc. of concentrated ammonia. After a thorough stirring the beakers

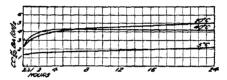


Fig. 4.—Graphs showing the production of amino nitrogen at different temperatures.

were allowed to stand overnight, and the contents were then filtered and the precipitate washed four times with 2 per cent ammonium hydroxid. The precipitate was then dissolved in \$0 cc. of dilute (1:4) nitric acid and the filter washed with 100 cc. of hot water. The filtrate and washings were then boiled, in order to destroy organic matter, and the phosphorus determination was completed in the usual way. For convenience, this will be called inorganic phosphorus. Whether or not that is the case may be questioned, and the merits of this method of determination are not discussed here. We simply used this method as one best suited to our purpose, and the results obtained (Table IV) are used for their comparative value.

Treatment N

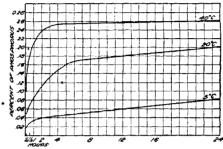
TABLE IV .- Determination of phosphorus in wheat extract

Tem- ! pera- ! ture. :		Total percent- age of phos- phorus in the extract.	
•с.	·	i	
5	5 minutes	0.018	0. 01
5	30 minutes		. 02
5	i hour	. 027	. 02
5	24 hours	. 083	. 07
20	5 minutes	. 0.14	. 03
20	30 minutes	. 003	. 03
20	1 hour	. 007	. 05
20	2 hours *.	. 100	. 0
20	4 hours	150	. 08
20	8 hours	. 173	. 00
20	16 hours	208	. 00
20	24 hours.	. 190	. 11
40	5 minutes	. 000	. 03
40	35 minutes	. 183	. 11
40	r hour	. 210	. 14
40	2 hours	.1 .240	. 17
40	4 hours	. 253	. 21
40	8 hours	., 254	. 22
40	ro hours	257	. 25
40	24 hours.	259	. 24
50	5 minutes	. 162	. 10
50	30 minutes.	. 215	. 15
50	t hour		
50	2 hours	. 254	. 22
50	4 hours	. 258	

- ). It will be noted that at  $50^{\circ}$  C, the total and inorganic phosphorus increases with the time of digestion.
- 2. At 20° C, the total and inorganic phosphorus increases with the time of digestion, but the total phosphorus does not show any increase after 16 hours. The ratio between the total and inorganic phosphorus varies considerably, but the latter averages a little more than one-half of the total.
- 3. At 40° C, the total and inorganic phosphorus increases with the time of digestion, but the maximum for both total and inorganic phosphorus is reached at about 4 hours. The proportion of inorganic phosphorus in relation to the total is much greater than at the lower temperature. After four hours the inorganic phosphorus is almost equal to the total.
- 4. At 50° C, the maximum of both total and inorganic phosphorus is reached in about two hours. The results are graphically presented in figures 5 and 6.

Why does hydrogen-ion concentration remain the same or show no increase with the increased time of digestion, while the amount of  $N/z_0$  barium hydroxid necessary to bring the solution to the concentration

 $P_{\pi}$  7,  $P_{\pi}$  8.3, or  $P_{\pi}$  9.3 shows a constant increase corresponding with the time of digestion, and also an increase in the total and inorganic phosphorus? The only explanation that we have to offer at this time is that the extract of wheat contains a definite amount of the hydrogen ion,



F10. 5.—Graphs showing the total phosphorus in wheat extractat different periods of extraction and at different temperatures.

and that this amount is not increased during the hydrolysis of the wheat because the phosphates which are produced during digestion are of such a nature that they undergo very small ionization in the water. When, however, the hydroxid is added, they undergo ionization. The percentage of ionization in these phosphorus compounds must be very small.

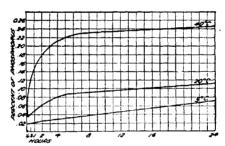


Fig. 6.—Graphs showing the inorganic phosphorus in wheat extract at different periods of extraction and at different temperatures.

#### SUMMARY

- (1) This paper presents the results of a study in determining, by means of the hydrogen electrode, the different hydrogen-ion concentrations in the extract of ground wheat. The total phosphorus and the phosphorus precipitated with magnesia mixture were also determined in the extract.
- (2) Extractions were made at the following temperatures: 5°, 20°, 40°, and 50° C.; and for the following periods: 5 and 30 minutes; 1, 2, 4, 8, 16, and 24 hours.

- (3) The temperature at which the extraction was made was found to have but little influence upon the hydrogen-ion concentration. The higher temperatures give an extract of but slightly higher concentration.
- (4) The duration of the digestion period did not influence the hydrogenion concentration. The average hydrogenion concentration when the extraction was made for 5 minutes, 30 minutes, and 1 hour was the same as when the extraction was made for 8, 16, and 24 hours.
- (5) But while the hydrogen-ion concentration of the extract shows no increase with the duration of the digestion, the quantity of N/20 barium hydroxid necessary to add in order to change the concentration to a definite point was greater in amount and within certain limits proportionate to the duration of the digestion.
- (6) The substances produced when wheat is digested in water are not ionized until an alkali has been added. The amount of these substances produced bears a definite relation to the time and temperature used in digestion. A limit, however, is soon reached, and this limit is reached sooner at the highest temperature.
- (7) The amino nitrogen as determined by the Sörensen formaldehyde method is all extracted in two hours at 40° C.
- (8) At 20° C, the amount of phosphorus in the extract precipitated by magnesia mixture averages about half of the total. At 40° practically all of the total phosphorus is converted into forms that are precipitated by the magnesia mixture.
- (9) The hydrogen-ion concentration of the water extract of wheat is definite in amount. This concentration is not changed during the extraction in proportion to the time. The reason for this is that the conditions for ionization are not present until an alkali is added. When, however, this is added, ionization takes place and the amount of standard alkali necessary to add in order to lower the hydrogen-ion concentration to a given point bears a proportionate relation to the temperature and duration of the digestion period.

#### ASH ABSORPTION BY SPINACH FROM CONCENTRATED SOIL SOLUTIONS

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#### INTRODUCTION

In 1915 when the authors were engaged in a study of the possible causes of spinach-blight, the theory was advanced that the disease was a form of "malnutrition" due, in a measure, to the accumulation of an excess of fertilizer salts in the soil solution

#### FERTILIZER SUBSTANCES USED

In the hope of reproducing the symptoms seen, beds of spinach (Spinacia olcracea) grown on the grounds of the Virginia Truck Experiment Station, in cooperation with which this work was carried on, were given large applications of the commoner fertilizer salts, singly and in the usual mixtures. The so-called "acid mixture" was that generally employed by the truck farmers of the Norfolk region for use in their spinach fields. The "basic mixture" was made up of substances which would likely be neutral or basic in the soil. The constituents of each were approximately as follows:

ACID: MIXYURE	BATE MINTURE.				
13	ounds.		Pounds.		
Ammonium sulphate	3.46	Sodium nitrate	450		
Acid phosphate	830	Basic slag from Birmingham, Ala	. 720		
Potassium muriate	170	Potassium sulphate	. 170		
Dried blood	200	Dried blood	260		
Tankage	400	Tankage	.100		

Single salts were supplied in two proportions: One was intended to be near the maximum; the other to cause clear injury. The substances applied are listed below in terms of pounds per acre:

SUBSTANCES.	QUANTITY USED.
Calcium carbonate	3 and 6 tons per acre.
Magnesium carbonate	
	750 and 1,500 pounds per acre.
Sodium nitrate	Do.
Sodium chlorid	Do.
Sodium sulphate	

<sup>1</sup> The authors are indebted to the Director of the Virginia Station and to his assistants for help in many ways. To Mr. J. A. McClintock, at that time Plant Pathologist of the Station, they owe an especial debt for careful notes made from time to time and for help rendered in other ways.

Journal of Agricultural Research, Washington, D. C.

SUBSTANCES—continued.	QUANTITY USED.
Acid phosphate	1,000 and 2,000 pounds per acre.
Complete mixture:	
Acid	2,000 and 4,000 pounds per acre-
Basic	Do.
Stable manure	20 and 40 tons per acre.

Control plots alternated with those receiving treatment.

The land used for planting had not been used for spinach for many years previously and was in good condition. It had received excellent treatment for several years and seemed to be very uniform in all respects.

Each bed was 5 feet wide and 61 feet long, and received four rows of seed. The chemicals were applied on July 29, 1915, and immediately hoed into the soil. The land was kept free of weeds until September 11, when curled Savoy spinach seed was drilled in. In 10 days the stand could be reasonably well determined, and since the variation with the treatment was clearly seen, the result may be summarized here.

#### PROCRESS OF PLANTS IN THE FIELD

The "best" stand was seen in the beds receiving stable manure; "very good" in beds receiving magnesium carbonate; "good" in those with calcium carbonate, acid phosphate, sodium sulphate, and basic complete mixture; "scattering" only in those with potash, sodium nitrate, sodium chlorid, and complete acid mixture. The controls were in the class designated "Good," a few in "Very good."

Owing to the unsatisfactory stand in some of the beds, all were reseeded on September 23, and irrigated. On September 30 plants began to appear, a thick stand being seen by October 6. The usual cultivation and thinning took place about a fortnight later.

From notes taken on the beds as they appeared on October 27, certain outstanding features may be developed. On taking completeness of stand, growth, and color as criteria, the treated plots were distributed in the following groups:

Excellent: basic complete mixture.

Very good (equal or better than the best control plots): acid phosphate, sodium sulphate (heavier treatment), magnesium carbonate, manure.'

Good (equal to the poorer control plots): calcium carbonate, sodium sulphate (lighter treatment).

Poor (poorer than controls): complete acid mixture, sodium chlorid, potash, sodium nitrate.

The poor plots were marked by a yellowish-green color, poor growth, and death of some of the seedlings, giving a bad stand. The greatest injury was seen in those parts of the plots receiving the heavier treatment,

Another review of the plots was made on December 1, 1915, with similar results.

The "best" plot in the whole experiment was that receiving the basic complete mixture.

Those having acid phosphate and sodium sulphate were "excellent."

"Good" would be said of plots receiving magnesium carbonate; somewhat less so, were those receiving calcium carbonate, which gave a very deep green color, and manure, especially the part of the bed receiving the lighter application.

The four plots found to be "poor" were those having sodium chlorid, sodium nitrate, and acid complete mixture. Poorest of all was potash. All poor plots were alike in having a crusted soil surface, with a suggestion of moisture.

At this stage samples were taken from several of the beds for ash analysis at Washington.

#### ASH DETERMINATIONS

The plants, after being divided into roots and tops, were asked in an electric furnace at a low red heat of approximately  $6\infty^{\circ}$  C. The total ash being determined, the chief constituents were worked out by the methods recommended by the Association of Official Agricultural Chemists.

The results are given in Table I. In the first section the results are calculated as percentages of the air-dry weight of the plant material, while in the second section the individual constituents are calculated as percentages of the total ash.

Table I.—Ash constituents of spinach calculated as percentages of dry material.

Fertilizer.	Sodium nitrate.			Sodium chlorid.		Sælium sulphate.		Potassium chlorid.		Calcium carbonate.		Control.	
	Tops.	Roots	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Торя.	Roots.	Tops.	Roots.	
Total ash	19-94	8.87	21.81	R. 58	20-70	8.30	17.67	7-40	21- 39	9.40	10-68	8.01	
Silica (SiO <sub>2</sub> )	4-50	1-77	3 80	1- 46	4-72	1.61	4- 30	1 - 48	7-97	3.16	5- 79	1.48	
(Mn <sub>2</sub> O <sub>4</sub> )	-035	- 14	. 01	-14	.02	- 645	.03	.05	-03	. 07	.025	. 06	
Lime (CaO)	1.11	- 29	1.13	• 45	.83	- 30	- 94	.30	J. 26	-45	1.14	. 30	
Magnesia (MgO)	1.13	.47	1.44	- 55	1.33	-41	1.68	- 56	1.14	-49	1.38	. 32	
Potash (K.O)	5.22	3-25	3.68	1.94	9.11	2.91	6.56	2.44	7.20	2.97	8-60	3.4	
Soda (NarO) Sulphur trioxid	3.50	- 86	5-67	1.42	.80	1.28	1.53	- 54	1.70	- 45	.91	- 33	
Phosphorus pent-	-44	.21	- 47	. 23	- 76	.32	-44	- 15	-47	. 23	52	- 33	
oxid (P <sub>2</sub> O <sub>2</sub> )	1.03	-92	1.22	1.16	1.20	1.11	1.11	- 98	.98	1.01	Z- 31	.84	
Alumina (Al <sub>2</sub> O <sub>3</sub> )	·61	- 30	. 57	- 20	-62	- 74	- 49		. 53	.45	. 63	- 20	
Perric oxid (Fe <sub>2</sub> O <sub>2</sub> ).	- 13	. 07	- 09	- 06	80 .	. 26	. 07	.05	.11	.07	.09	. 00	
Total	17.705	8- 28	18-09	7-61	19,56	8.305	17-17	6.73	21.38	9-37	20.415	7-40	

<sup>1</sup> Wiley, H. W., ed. official and provisional methods of analysis, association of official agricultural chemists, as compiled by the committee on revision of methods. U. S. Dept. Agr. Bul. 107 (rev.), 271 p., 13 fig. 1968.

<sup>92801°-19-2</sup> 

## TABLE I.—Ash constituents of spinach—Continued CALCULATED AS PERCENTAGES OF DRY MATERIAL—continued

Fertilizer.	Acid phos- phate.		Complete acid.		Complete basic.		Manure.		Average.	
	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.
Total ash	22.39	13.40	20. 59	10-48	18. 6a	10.01	21.73	11.40	20.45	9. 58
Silica	7-14	5-43	5-40	2.78	4- OT	2.31	4.47	2-47	5. 21	2.39
Manganous oxid ! Lime	- 04 I- 13	.38	.05	.05	.03	.04	- 84	. 28 - 49	1.031	. 38
Magnesia	1.33	-49	1.33	-56	Z. 34	.75	1.59	.69	1.37	53
Potash	6.40	1.76	8.69	3.36	8-70	3.97	9.76	4.56	7-39	3.06
Soda	2.15	.99	- 78	1.58	-66	- 40	•45	- 70	1.82	-85
Sulphur trioxid Phosphorus pent-	- 53	. 28	- 58	- 35	.53	.31	-48	-40	. 52	- 28
oxid	1.47	. 84	1.12	1.26	1.32	1.49	1.08	1.36	1.20	1.10
Alumina	- 70	-40	.65	-33	• 45	-32	· 51	. 36	- 58	• 30.
Ferric oxid	- 09	.08	•10	.08	•10	.09	- 11	. 15	-097	•07
Total	20-98	10-74	19.66	10-78	18.11	10.08	19-306	11-46	19. 247	9.06

#### INDIVIDUAL CONSTITUENTS CALCULATED AS PERCENTAGES OF THE TOTAL ASH

Total ash 19.94 8.87 21.81 8.58 20.70 8.30 17.67 7.40 21.39 9.40 20.68 8  Silica 22.57 19.93 17.42 17.02 22.80 17.04 21.30 17.00 7.40 21.39 9.40 20.68 8  Manganous oxid 21 5.57 3.77 5.18 5.24 4.01 3.50 5.23 4.05 5.89 4.89 5.51 3.40 Manganous 2.50 7.30 6.00 41.59 4.0	Fertilizer.		Sodium nitrate.		Sodium chlorid.		Sodium sulphate,		Potassium chlorid.		Calcium carbonate.		Control.	
Silica	refunzei.	Tops.	Roots.	Tops	Root	s. Tops.	Roots.	Tops.	Roots	. Tops	Roots	Tops.	Roots.	
Manganous oxid	Total ash	19.94	8.87	21.81	8.5	30-70	8.30	17-67	7-40	21.39	9-40	20.68	8. qz	
Potash	Manganous oxid Lime	. 18 5- 57	1-58	5.18	1 · 6	4 4.01	3.6r	- 17	4.05	5.89	4.89	5.51	18.48 • 75 3• 75	
Oxid	Potash Soda Sulphur trioxid	26. 18 17. 55	36.64 9.70	16.87 26.00	22.6 16.5	1 44 OI 5 3 82	35.06 15.42	37-13 8-66	32 97 7-30	33-66	31.60	41.59 4.40	4- 24 42- 95 4- 12 4- 12	
Acid phosphate.   Complete acid.   Complete basic.   Manure.   Average.	exid	3.06	3.38	2.61	2.3	3.00	2.89	2.77	2.43	2.43	4.89	3.05	9.95 2.25	
Pertilizer.   Tops.   Roots.   Tops.   T	Total	88.91	93.36	82.88	88.6	94-41	100-05	97.25	90.9	99-65	100,00	98-72	91.40	
Total ash 21.30 13.40 20.59 10.48 18.66 10.01 21.73 11.40 20.45 5  Silica 33.35 40.52 26.33 26.53 21.56 23.08 20.57 21.66 28.48 2.26 21.40 21.20	Fertilizer.									Manu	re.	Ave	age.	
Silica   33.35   40.52   26.23   26.53   21.56   23.08   20.57   21.66   25.48   24.66   24.66   25.28   26.23   26.		Tops.	Roo	ts.	lops.	Roots.	Tops.	Roo	ts. T	ops.	Roots.	Tops.	Roots.	
Mananous oxid         -ip         -67         -24         -43         -if         -40         -07         2.46         -i4         -14	Total ash	21.39	1 r3.	40	20. 59	10.48	18-60	10.	DI 2	1.73	11.40	20-45	9. 5	
Magnesia.     6. 22     3. 66     6. 6 5. 34     7. 20     7. 49     7. 32     6. 56     6. 70       Potash.     29-92     13-13     42-20     32-06     46-77     39-66     44-91     46-00     36-14     38-36       Soda.     10.05     7-39     3-79     15-08     3-53     4-00     2-07     6-14     8-90     8       Sulphur trioxid.     2-86     2-09     2-82     3-34     2-88     3-30     2-21     3-51     2-54       Phosphorus     6-87     6-87     5-44     12-02     7-10     14-89     4-97     11-05     5-87     11       Alumina     3-27     2-99     3-16     3-15     2-42     3-20     2-55     3-10     2-84	Manganous oxid	. 19	) ·	67	- 24	. 48	- 16	1	40	.07	2-46	- 14	24·9 1·0 3·9	
Sulphur trioxid 2.48 2.09 2.82 3.34 2.85 3.10 2.21 3.51 2.54 2.76 2.76 2.76 2.76 2.76 2.76 2.76 2.76	Magnesia Potash Soda	29.9	3. 13.	66 13	6.46 42.20	5.34 32.06	7. 20 46. 77	39.	49 66 4	7- 32 4-91	40.00	6. 70 36. 14	5 · 5. 31 · 9. 8 · 8	
	Sulphur trioxid Phosphorus pent	2.48	1	1	ļ	-	1 -	1	- 1	İ		5-87	2.9	
													3 1	

In order to bring out more clearly the relationships involved in Table I, graphs have been prepared in which quantities are indicated on a uniform scale. In figure 1 appear the values found for the tops, and in figure 2 similar values for the roots. Each unit on the perpendicular axis represents 0.1 per cent of dry weight in every case.

#### TOTAL ASH

A casual inspection of these results reveals the fact that the total ash content of the tops calculated as percentage of dry weight, while showing considerable variation, is always greatly in excess of that of the roots, in obedience to the general rule. As regards the influence of specific substances on the total ash absorption certain coincidences may be noted. The total ash reached its minimum in both roots and stems in the plot treated with potash. It is depressed in the tops nearly as much in the presence of the basic mixture, to a less degree in the roots. In general, the quantity of ash constituents is less for the roots in the plots treated with sodium salts and in the untreated control than in the other plots.

Of the single salts calcium carbonate alone goes with a total ash in the roots, approaching that seen in the mixtures of several salts. This depressing action of the sodium salts is not seen in the ash content of the tops. Acid phosphate goes with the highest total ash seen in any sample of roots; sodium chlorid and manure, with the highest totals seen in the tops.

#### INDIVIDUAL ASH CONSTITUENTS

In examining the quantities of the different ash constituents seen in Table I it will be noted that in some cases there is a great variation among the different plots; in others little difference is to be seen. Those showing great variation are silica, potash, and soda; those showing little change with the change in outside conditions are lime, magnesia, phosphorus pentoxid, sulphur trioxid, manganous oxid, alumina, and ferric oxid. It seems as though all plants were able to absorb these from the soil to a point of steady equilibrium without much regard to the substances offered. It appears that even when an excess of any of the ions present in this latter group of compounds is present no considerable increase in the absorption of these ions takes place.

The quantity of ions absorbed from the group of variable salts seems much more subject to influence from the added salts. In some cases the ions present in excess are themselves absorbed in greater quantity. This seems to be the case with sodium-nitrate and sodium-chlorid plots, in which considerably greater quantities of soda appear in the ash of the tops than in any other plots. Sodium sulphate, however, gives not

<sup>1</sup> PALLADIN, W. PFLANZENPHYSIOLOGIE. Bearb, aufgrund der 6. russischen aufl. p. 83. Berlin, 1911.

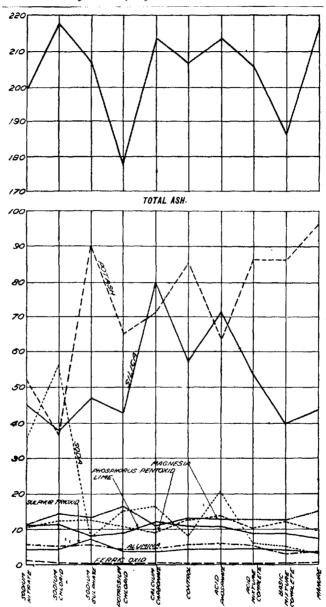
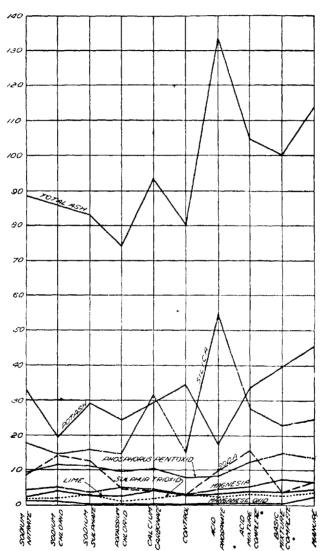


Fig. 1.—Graphs representing total ash and individual ash constituents found in spinach tops from plots receiving the substances indicated.



Pro. 2.—Graphs representing total ash and individual ash constituents in spinach roots from plots receiving the substances indicated.

such result. The greater absorption of sulphur trioxid occurs in the only plot treated with great quantities of sulphates, but the increase is not great.

In some cases a marked increase in a given constituent accompanies the presence in excess of some other ion. This is seen in the plot treated with sodium sulphate, in which potassium absorption in both root and top is high. A similar result is seen in the plots treated with stable manure and with both acid and basic complete mixtures. On the other hand, potassium absorption is decreased in the acid-phosphate plot in both tops and roots. Potassium chlorid in excess also accompanies a reduction of potash in the ash of both parts of the plant.

Silica is greatest in plots receiving calcium carbonate and acid phosphate, lowest in those dosed with sodium salts, and in the control plot.

It is interesting to note that, although the manganous oxid content when referred to dry weight is small in all cases, it is consistently higher in the roots than in the tops, acid complete mixture excepted, this being the only constituent which is not more abundant in the tops than in the roots.

#### RATIOS BETWEEN PAIRS OF CONSTITUENTS

In a number of cases there seemed to be some evidence that a roughly reciprocal relation exists between pairs of constituents. This tendency was most marked in the plots receiving mixtures of salts. Thus, in general, when silica was high, potash was low in both tops and roots.

The silica-potash ratios, as worked out for the different plots, are summarized in Table II.

	Potash (K2O).			
Pertilizer.	Tops.	Roots.		
	*			
Sodium nitrate	1. 16	1. 84		
Sodium sulphate	1.93	1. 8i		
Sodium chlorid	. 95	1. 33		
Potassium chlorid	1. 53	1.65		
Calcium carbonate	. 90	. 94		
Acid phosphate	. 90	. 32		
Complete acid	1. 61	1. 21		
Basic complete	2. 17	1. 72		
Manure	2. 19	1.85		
Control	T. 40	2. 22		

TABLE II.-Silica-potash ratio in ash of spinach plants. Silica (SiO2)=1

It will be noted that, while both of these constituents belong to the group of the more variable ones, the ratio is less variable; silicia being unity, potash lies between 1.00 and 2.00 in a majority of cases. In plots receiving heavy doses of calcium-containing fertilizers (calcium carbonate and acid phosphate) as well as in the tops from the sodium-chlorid plot, the silica exceeds the potash. Again, potash has high

relative values, especially in the tops, in the plots to which the complete fertilizers were applied.

The relation of potash and soda is of particular interest in view of the halophytic nature of the spinach plant. It is perhaps worth noting in this connection that the sodium-potassium ratio varies over a wider range, something to be expected perhaps in view of the greater variability in the quantities of these constituents present. In Table III these ratios are given, soda being unity, the values of potash appearing in the appropriate columns.

TABLE III.-Soda-potash ratio in spinach ash. Soda (NaO4)==1

the state of the s		
	Potash (	$K_7O)$ .
Fertilizer.		
+	Tops.	Roots.
Sodium nitrate	1. 49	3. 78
Sodium sulphate	11. 53	2. 27
Sodium chlorid	. 65	1. 37
Potassium chlorid	4. 29	4. 52
Calcium carbonate	4- 24	6. 46
Acid phosphate	2. 98	1, 78
Acid complete	11. 14	2. 13
Basic complete	13. 18	9. 02
Manure	21. 70	6. 5 <b>r</b>
Control	9-45	10. 43

It will be seen that in a broad way, when the potash is high in the ash, soda is low, and vice versa. Potash, however, is always higher than soda, the excess being greater in the leaves than in the roots. High potash seen in the plot receiving sodium sulphate is accompanied by a very low soda content. The same is seen in the control as well as in both acid and basic complete mixtures and in stable manure. High soda is seen in the plots treated with sodium chlorid, in which potash reaches its minimum, and in that receiving sodium nitrate. The control culture and that receiving acid phosphate show the same less strikingly. Potash is in greatest relative excess in the plots receiving the complete fertilizers.

These results suggest the possibility that sodium may be able to perform some functions in the plant which are usually performed by potassium. It seems likely that by giving proper mixtures of the alkalis it might be possible without detriment to the plant to get along with less of the expensive potassium constituent, thus protecting the potassium in the soil.

In view of the fact that calcium is usually found in the ash of a great majority of plants in considerably greater quantity than magnesium it is of interest to note the quantities found in these spinach plants.

 $<sup>^{-1}</sup>$  Loew, Oscar. Liming of soils from a physiological standpoint. In U. S. Dept. Agr. Bur. Plant Indus. Bul. 1, p. 9–35. 1997.

It appears from data scattered through Wolff's <sup>1</sup> tables that in some cases the magnesium content exceeds that of calcium, especially in the beet family. In the leaves of these plants the calcium usually much exceeds magnesium, while the reverse holds for the roots. In the analysis given by Wolff <sup>2</sup> lime exceeds magnesia in spinach in an approximately 2 to 1 ratio. In Table IV the calcium-magnesium ratio is shown for each experimental plot.

TABLE IV.—Calcium-magnesium ratio in ash of spinach plants. Lime (CaO)=1

	Magnesia (MgO).	
Fertilizer.	Tops.	Roots.
Sodium nitrate Sodium sulphate Sodium chlorid Potassium chlorid Calcium carbonate Acid phosphate Acid complete Braic semplete	1. 02 1. 60 1. 27 1. 79 . 90 1. 18 1. 39	1. 62 1. 37 1. 23 1. 87 1. 07 1. 29 1. 30 1. 87
Basic complete. Manure Control	1. 40 1. 89 1. 21	1. 67 1. 41 1. 13

It will be noted that magnesia exceeds lime in every case, regardless of the nature of the substances applied, except in that of the tops in the plot receiving lime, in which the lime exceeds the magnesia. Spinach seems to be even a more pronounced user of magnesium than the sugar beet. The field notes show that the plot receiving magnesium carbonate was somewhat better than that receiving calcium carbonate. Unfortunately no sample from the magnesium-carbonate plot was ashed.

#### SUMMARY

Spinach plants grown on the grounds of the Virginia Truck Experiment Station at Norfolk in beds given heavy treatments of fertilizer salts, singly and in mixtures, gave best results in plots receiving a complete mixture having a basic or neutral character in the soil (sodium nitrate, basic slag, and potassium sulphate); next best with acid phosphate and with sodium sulphate; poor in plots receiving heavy treatments of sodium chlorid, sodium nitrate, and acid complete mixture (1 to 2 tons per acre); poorest with potassium chlorid.

A study of the ash showed the highest total ash in the tops in plots with sodium clillorid, calcium carbonate, acid phosphate, and manure; lowest with potassium chlorid and basic complete mixture. The highest ash was in roots accompanied with acid phosphate and manure, the lowest with potassium chlorid and sodium salts. General excellent condition of

WOLFF, E. ASCHEN-ANALYSEN. T. 2, p. 42-50. Berlin. WOLFF, E. OF. CIT, p. 128.

the crops does not parallel high ash absorption, the best and poorest plots having plants with low ash.

Ash constituents fall into two groups: (1) those present in quantities that show relatively little variation whatever be the chemicals added to the soil—lime, magnesia, phosphorous pentoxid, sulphur trioxid, manganous oxid, alumina, and ferric oxid; and (2) those which show great fluctuations in the quantity present—silica, potash, and soda.

In the first group the plants seemed to be able to get the required quantity of constituents mentioned from the soil of all plots studied whatever was offered in excess, and reached an equilibrium that was little affected by the varying conditions.

In the second group wide variations occur, sometimes with an increase of the ions offered in excess, as in sodium chlorid and sodium nitrate, sometimes by the absorption of something else, as increase in silica in plots receiving calcium carbonate and acid phosphate.

Manganous oxid is the only constituent regularly present in greater proportion in the roots than in the tops.

In some cases the high absorption of one constituent is accompanied by the low absorption of another, and vice versa. Such reciprocal pairs are silica and potash, soda and lime, and potash and magnesia. The silica-potash ratio is relatively steady. When silica equals 1, potash varies between 1.16 and 2.18 in the tops and between 1.33 and 2.32 in the roots, except when the substance added to the soil is high in calcium, when the value of potash becomes less than unity in both tops and roots.

The soda-potash ratio is much more variable, being always more than in both tops and roots. When mixtures of salts are added to the soil, potash rises to very high relative values.

There is a suggestion that sodium may perform some functions also performed by potassium, indicating the possibility that sodium might in part replace potassium in fertilizers.

The calcium-magnesium ratio in spinach, both in leaves and in roots, is exceptional in having a value greater than unity. The only exception is seen in the tops of plants receiving a heavy treatment with calcium carbonate. This fact seems to suggest the practical importance of magnesium salts as fertilizers for spinach.

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